

We claim:

1. A nucleic acid enzyme capable of recognizing and cleaving a nucleic acid substrate at a cleavage site which when bound to the substrate comprises:

5 (a) a substrate binding portion base-paired to the 6 nucleotides 3' to the cleavage site of the substrate and which binding portion comprises the sequence:

*SB*  
*SB*  
3' -UNNXNN-5'

wherein each

10 N is a nucleotide which may be the same or different, and

X is a nucleotide selected from the group consisting of T, U, A, G;

15 (b) a region P3 comprising a double-stranded portion bound covalently at a bottom end to the remainder of the ribozyme and capped at a top end by a loop L3;

(c) a region P2 comprising a double-stranded portion bound covalently at a bottom end to the remainder of the ribozyme;

20 (d) a region P4 comprising a double-stranded portion bound covalently at a bottom end to the remainder of the ribozyme, wherein the first base-pair at the bottom end of P4 is a homopurine base-pair;

25 (e) a double-stranded region P1.1 formed by base-pairing two nucleotides located between the substrate binding portion and the P4 region, with two nucleotides in the L3 loop; and

(f) a single-stranded region, J4/2, covalently bound at one end to the bottom end of P2 and covalently bound at the other end to the bottom end of P4.

2. The nucleic acid enzyme according to claim 1, wherein the base-pair at the bottom end of the P3 region is not 3'-C-G-5'.

3. The nucleic acid enzyme according to claim 2, wherein the 5 first base-pair at the bottom end of the P3 region is selected from the group comprising U-A, A-U, T-A, and A-T.

4. The nucleic acid enzyme according to claim 1, wherein the base-pair at the bottom end of the P3 region is 3'-U-A-5'.

5. The nucleic acid enzyme according to claim 1, wherein the 10 double-stranded portion of P2 is not capped at a top end.

6. The nucleic acid enzyme according to claim 5, wherein the first three base-pairs at the top end of the double-stranded portion of P2 are G-C or C-G base-pairs.

7. The nucleic acid enzyme according to claim 1, wherein the 15 first three base-pairs at the top end of the double-stranded portion of P2 are 5'-G-C-3' base-pairs.

8. The nucleic acid enzyme according to claim 1, wherein the P1.1 stem is comprised of two GC base pairs.

9. The nucleic acid enzyme according to claim 1, wherein the 20 J4/2 strand is at least 5 nucleotides long.

10. The nucleic acid enzyme according to claim 1, wherein the J4/2 strand is 5 nucleotides long.

11. The nucleic acid enzyme according to claim 1, wherein the double-stranded portion of the P4 region comprises the sequence 5'-GCAUSG-3' or 5'-GCAUSSG-3', wherein S is G or C.

12. The nucleic acid enzyme according to claim 1, wherein the L3 loop consists of 7 or fewer nucleotides.

13. The nucleic acid enzyme according to claim 1, wherein the nucleic acid enzyme is derived from antigenomic hepatitis

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delta virus.

14. The nucleic acid enzyme of claims 1, wherein the substrate binding portion of the enzyme comprises the sequence 3'-UNNXNNN-5'.

5 15. The nucleic acid enzyme of claim 14, wherein the substrate binding portion of the enzyme comprises the sequences 3'-UNNANNN-5' or 3'-UNNGNNN-5'.

*Sub 37*  
16. The nucleic acid enzyme of claims 1, wherein the enzyme is composed of ribonucleotides.

10 17. The nucleic acid enzyme of claims 1, wherein the enzyme is composed of a mixture of ribonucleotides and deoxyribonucleotides.

15 18. A method for cleaving a nucleic acid substrate with a nucleic acid enzyme at a cleavage site comprising mixing the nucleic acid enzyme according to claim 1 with the substrate, wherein

the substrate includes a 7 nucleotide sequence with at least 6 nucleotides 3' to the cleavage site and at least 1 nucleotide 5' to the cleavage site and of formula:

20  $5' - H' \downarrow GNNHNN - 3'$

wherein each

N is a nucleotide which may be the same or different,

H is a nucleotide selected from the group consisting of A, U, C, and T,

25  $\downarrow$  is the site of cleavage, and

H' is a ribonucleotide selected from the group consisting of A, U, and C,

wherein

*Sub B*  
5 (i) the first nucleotide 3' to the cleavage site is capable of forming a wobble pair with the enzyme,

10 (ii) the second, third, fifth, and sixth nucleotides 3' to the cleavage site are capable of forming conventional Watson-Crick base pairs with the enzyme,

15 (iii) the fourth nucleotide 3' to the cleavage site is capable of forming a triplet with the enzyme comprising a non-conventional Watson-Crick base pair and a conventional Watson-Crick base pair, and

10 (iv) the ribonucleotide directly 5' to the cleavage site does not form a base pair with the enzyme.

19. The method of claim 18, wherein in the substrate, H' and a nucleotide immediately 5' to it are not both pyrimidine nucleotides.